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DISTRIBUTION OF AFLATOXINS IN CORN FRACTIONS VISUALLY SEGREGATED FOR DEFECTS

Fabiana Segatti Piedade[1]; Homero Fonseca[2]*; Eduardo Micotti da Gloria[3]; Maria Antonia Calori-Domingues[3]; Sônia Maria Stefano Piedade[4]; Décio Barbin[4]

[1]Ciência e Tecnologia de Alimentos, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brasil. [2]Departamento da Produção Vegetal, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brasil; [3]Departamento de Agroindústria, Alimentos e Nutrição, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brasil; [4]Departamento de Ciências Exatas, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brasil.

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ABSTRACT

The aflatoxin distribution in corn fractions obtained after visual segregation for defects in 30 samples, known to be contaminated, was studied. Each sample was passed through a 5.0 mm round holes sieve, graded for defects and then segregated in sound kernels (regular kernels) and non-sound kernels (injured, germinated, fermented, moldy, heated, insect damaged, immature, broken, hollow, fermented up to ¼, discolored, extraneous materials, and injured by other causes), as defined by the Brazilian Official Grading rules for corn. The non-sound kernels showed the highest contamination levels in all samples. The contamination levels of non-sound kernels (20% of total weight) ranged from 23 to 1,365 µg/kg of aflatoxins (B_1, B_2, G_1 and G_2) and were higher than sound kernels ($p<1\%$) ranging from not detected (ND) to 126 µg/kg and in 87% of these the aflatoxin contents were lower than 20 µg/kg. Statistically significant correlation indexes were found among the percentage of defective groups like fermented, heated and sprouted kernels or the total injured kernels, and the estimated contamination levels for the sound and non sound fractions. It was concluded that the non-sound kernels fraction, even being small in weight, has contributed with 84% of the estimated contamination of the samples. The segregation of the non-sound kernels would favor a reduction in the contamination of corn lots. The poorer quality corn types (types 3 and Bellow Standart) have predominated among samples of the experiment.

Key words: aflatoxins, corn, contamination distribution, visual segregation, fraction.

INTRODUCTION

Corn is a basic ingredient of human and animal feeding. Due to its nutritional composition it is a good substrate for fungi development that may cause nutritional losses and production of toxic substances known as mycotoxins (8).

Corn follows peanuts, as the most reported aflatoxins contaminated commodity (9). Several researches on aflatoxin contamination in corn were published in Brazil. Fonseca *et al.* (5,6), in a two-year survey with samples taken from supermarkets in the State of São Paulo, reported the presence of aflatoxin in 4.7% of corn samples, with a maximum contamination level of 2,000 µg/kg. Sabino *et al.* (11) also found that 10.4% of corn samples were contaminated. In a survey of 238 corn samples destined to poultry and swine feed, in Southern Brazil region (12), it was found that 28.5% were positive for aflatoxin. Gloria *et al.* (7) analyzed in the State of São Paulo, 292 corn samples taken from truckloads in the arrival at a wet-milling plant and found that 33.6% of them were aflatoxin positive. Santúrio *et al.* (13), analyzed 1,263 samples of corn and 1,006 samples of corn based rations, coming from several Brazilian States, in a period of ten years.

* Corresponding author. Mailing address: Rua Dona Eugênia, 269 – 13416-218, Piracicaba, SP, Brasil. E-mail: hfonseca@merconet.com.br

They found that 645 samples of corn and 468 samples of rations were positive for aflatoxins, with a maximum level of 14.4 and 5.1 µg/kg, respectively.

The contamination of agricultural commodities with mycotoxins is not uniform and very often only a few highly contaminated kernels may be present in a lot. That is the reason for the high variation in mycotoxin determination in samples of the same lot, and this fact turns difficult the determination of the actual concentration of these mycotoxins in a lot.

Some literature data have showed that probably aflatoxin contamination is concentrated in specific corn kernel types. According to Christensen and Kaufmann (4), injured and broken kernels are more susceptible to fungal attack than whole kernels. Rambo *et al.* (10) have observed a higher incidence of *Aspergillus flavus* in injured kernels. Shotwell *et al.* (14) have studied the aflatoxin distribution in corn lots and observed that large pieces of corn broken kernels (>4.5 mm) and injured kernels were highly contaminated. Afterwards, Shotwell *et al.* (15) observed that discolored corn kernels, although apparently sound, also presented high aflatoxin contamination and Shotwell *et al.* (16) verified that corn fractions that presented insect damage, discoloration, injured and broken were more contaminated with aflatoxins.

On the other hand, aflatoxin contamination was found in whole and apparently sound kernels in 10 lots of aflatoxin contaminated corn (3).

The aim of this study was to verify the distribution of aflatoxin in fractions, obtained after the visual separation of contaminated corn samples in sound and non-sound kernels.

MATERIALS AND METHODS

Sampling and sample treatment

Thirty samples of aflatoxin-contaminated corn were drawn from truckloads at their arrival in a food industry in the State of São Paulo, Brazil.

Two samplings were made in two periods and 16 samples and 14, respectively, were collected. Each sample was composed of several incremental samples taken from different points of the load, according to the industry's scheme, and then gathered to form one sample of, approximately 3 kg, considered representative of each load.

Every sample was previously screened for aflatoxins by the industry, according to the technique of the bright greenish-yellow fluorescence (BGYF) and if found to be positive, the contamination was confirmed by an Immunoaffinity Column Method (Aflatest, Vicam).

The samples were homogenized and passed through a sieve with 5.0 mm round holes to discard smaller materials.

After obtaining a representative sample, a portion of 250g was taken for grading, according to the Brazilian Official Grading rules for corn (Table 1).

Table 1. Tolerance levels of defects for corn grading in types, according to the Brazilian Official Grading rules for corn (1).

TYPES	TOLERANCES			
	Moisture (%)	Extraneous materials, Impurities and Fragments (%)	Injured (%)	
			Total	Maximum of Fermented and Sprouted
1	14.5	1.5	11	3
2	14.5	2.0	18	6
3	14.5	3.0	27	10
B.S.*	14.5	(To be specified in each case)		

*B.S. = Bellow Standard.

Visual grading was then carried out according to the following defective kernels definitions:

Fermented, heated and sprouted kernels - kernels or pieces of kernels that have lost the coloration or color characteristics, by action of heat and humidity or fermentation in more than 1/4 of the size of the kernel, and kernels or pieces of kernels that present visible germination.

Injured kernels - kernels or pieces of kernels, hollow, immature, attacked by insects, rodent or parasites, those fermented up to 1/4 of the kernel size, as well as injured by different agents.

Impurities, foreign materials and broken - fragments of the plant, as well as kernels or fragments of kernels that pass through a sieve of circular sieves of 5.0 mm diameter holes and the kernels or seeds of other species, as well as the vegetal remainings and extraneous materials of any nature, respectively.

After grading the samples were identified and stored in a freezer at -18ºC, in the laboratory, for further processing and analysis.

Each sample was passed through a 5.0 mm round holes sieves, and the fraction that passed through, plus the extraneous materials that did not pass through were taken out, remaining, in the sieve, only kernels. These kernels were then, hand picked selected and visually segregated in apparently sound kernels and kernels with some kind of injury or defect (non-sound kernels). The sound kernels were considered the regular kernels and the non-sound were those considered as fermented, heated and sprouted, injured (fermented up to ¼, broken, hollow, insect damaged), impurities, foreign materials and broken according to the Brazilian Official Grading of corn (1).

The sound and non-sound fractions were ground in a sampling mill (Romer Analytical Sampling Mill) and a subsample was ground in a hammer mill with a 20-mesh screen. Each fraction was homogenized and analyzed in duplicate for aflatoxins B_1,

B_2, G_1, and G_2, by TLC (17), with a detection limit of 1 µg/kg. The maximum accepted variation between duplicate results was 30%, otherwise the analysis was repeated. The total contamination of the sample was estimated considering the weight of each fraction and its respective contamination.

Statistical analysis

The statistical design of the experiment was the completely randomized with 30 repetitions. The contamination levels of the sound and non-sound kernels, considering the average of duplicate analysis, were compared to check if there were statistical differences between the fraction contamination levels by the ANOVA statistic test. For this, the data were transformed to log (x+0.5) before its utilization.

Correlations between contamination levels of sound or non-sound with the relative fraction participation in the sample were checked. Correlations between contamination levels of non-sound kernels and the percentage of injured or fermented kernels or impurities present were also checked.

RESULTS AND DISCUSSION

Grading by quality, showed a higher number of poorer corn types (type 3 and BS, with 10 and 13 samples, respectively) than better corn types, such as types 1 (3 samples) and 2, (4 samples), respectively (Table 2).

It was observed that the contamination levels of non-sound kernels ranged from 23 to 1,365 µg/kg of total aflatoxins (Table 3) and were statistically higher than in sound kernels p<1% (Tables 4 and 5), that ranged from not detected (ND) to 126 µg/kg. The contamination levels of 87% of the sound fractions were lower than 20 µg/kg, the maximum allowed aflatoxin level for corn in Brasil (2). In 13% of the samples the sound fractions had levels above the tolerance levels but with figures far lower than those of the non-sound kernels. On the other hand all samples of non-sound kernels were above 20 µg/kg.

The non-sound fraction weights varied from 8 to 35% of the total kernels of the evaluated samples (Table 3). The correlation between the percentages of the non-sound kernels with the aflatoxin contents was not statically significant (**r** = 0.25), suggesting that there is not a good correlation between these parameters.

The qualitative data (Table 2) of the samples in the groups: a) injured kernels, b) fermented and sprouted kernels, c) impurities and foreign material, showed correlation values (**r**) with estimated contamination levels of 0.51, 0.52 and 0.14, respectively. The statistically significant **r** values obtained for injured and fermented kernels, suggests a positive correlation between the percentage of these types of kernels and aflatoxin contamination. However, **r** values are low and considering the relatively small number of samples, this observation must be taken carefully and with some restrictions. On the other hand the **r** values obtained for impurities showed that they did not present correlation with the estimated contamination of the samples.

The participation of the sound kernels in the total weight of the samples was much higher (84%) than the non-sound ones (16%). Even that the sound kernels fraction has presented contamination levels as reported by other authors (3,15), they occurred in low levels with mean value of 15 µg/kg against the mean value of 271 µg/kg for the non-sound kernels (Table 3).

The data obtained in this experiment lead us to assume that the segregation of the non-sound kernels fraction, while in low

Table 2. Moisture content, percentage of defects present and grading by quality of corn samples, according to the Brazilian Official Grading rules (Brasil, 1976).

Sample	Moisture %	Injured (%)		Extraneous Materials, Impurities and Fragments (%)	Grading by type[a]
		Total	Fermented and Sprouted		
1	13.70	6.40	3.10	1.50	2
2	11.10	7.00	2.40	3.30	BS
3	13.70	8.20	3.10	2.40	3
4	13.40	9.50	3.40	3.00	3
5	13.90	5.00	3.30	1.40	2
6	12.70	9.50	6.60	3.20	BS
7	13.30	7.90	2.80	3.10	BS
8	12.20	7.90	3.60	4.00	BS
9	14.90	11.60	3.40	3.10	BS
10	12.40	8.20	2.60	1.90	2
11	12.60	10.00	1.90	1.50	1
12	13.20	7.40	4.00	2.50	3
13	13.20	10.30	3.10	3.40	BS
14	13.80	8.20	3.10	3.00	3
15	13.40	6.80	2.30	1.30	1
16	13.40	10.40	2.40	2.80	3
17	13.10	6.10	2.10	3.70	BS
18	12.00	11.90	3.90	3.30	BS
19	12.20	11.70	3.20	4.40	BS
20	11.80	7.40	1.40	2.00	2
21	12.80	10.00	2.20	3.50	BS
22	11.70	9.2	1.00	1.30	1
23	12.90	10.60	2.00	3.40	BS
24	12.70	10.40	1.30	2.70	3
25	12.40	20.40	4.40	3.10	BS
26	12.80	9.90	0.80	2.40	3
27	13.60	7.20	3.90	2.70	3
28	12.70	10.60	0.70	3.90	BS
29	12.70	11.20	2.70	2.70	3
30	12.40	17.10	7.20	2.60	3

[a] Grading of corn by quality in types 1, 2, 3 and BS, according to the percentage of defects observed in the lots (Table 1).

Table 3. Fraction weights, relative participation of fraction weigh in total sample weigh, total aflatoxins contamination (B1+B2+G1+G2) of fraction, relative participation of fraction contamination in total sample contamination level and total sample contamination and weight of corn samples.

Sample	Fraction A Sound kernels				Fraction B Non-sound kernels				Whole Sample	
	Weight (g)	(%)[2]	Cont.[1] (μg/kg)	(%)[3]	Weight (g)	(%)[2]	Cont.[1] (μg/kg)	(%)[3]	Weight (g)	Est. Cont.[4] (μg/kg)
1	1,471	83	33	47	301	17	183	53	1,773	58
2	1,008	86	N.D.	0	166	14	23	100	1,174	3
3	1,025	85	28	47	176	15	181	53	1,201	50
4	1,415	86	19	38	239	14	180	62	1,654	42
5	1,194	79	4	4	323	21	351	96	1,517	78
6	1,206	82	3	12	263	18	98	88	1,468	20
7	977	77	N.D.	0	291	23	346	100	1,269	79
8	1,100	81	7	27	259	19	80	73	1,359	21
9	987	78	10	21	285	22	128	79	1,272	36
10	924	82	5	14	208	18	136	86	1,132	29
11	1,347	83	4	16	280	17	100	84	1,627	20
12	1,087	80	12	3	280	20	1,365	97	1,366	289
13	1,161	83	3	26	243	17	41	74	1,403	10
14	1,222	73	4	5	454	27	186	95	1,676	53
15	1,090	84	5	9	207	16	271	91	1,296	47
16	948	80	8	24	231	20	104	76	1,180	27
17	1,225	78	5	16	338	22	94	84	1,563	24
18	1,172	80	10	7	291	20	538	93	1,463	115
19	1,032	68	104	23	480	32	742	77	1,512	306
20	1,327	82	6	14	286	18	169	86	1,613	35
21	1,190	80	4	5	296	20	277	95	1,487	58
22	1,315	84	8	14	242	16	262	86	1,557	48
23	1,178	81	N.D.	0	285	19	62	100	1,463	12
24	1,263	83	N.D.	0	254	17	84	100	1,517	14
25	895	63	126	35	535	37	391	65	1,430	225
26	1,218	84	4	37	239	16	35	63	1,457	9
27	1,101	77	8	18	333	23	120	82	1,434	34
28	1,117	79	12	14	291	21	276	86	1,408	67
29	1,250	81	3	3	298	19	376	97	1,548	75
30	926	65	14	3	501	35	918	97	1,427	331
Mean	1,146	80	15	16	296	20	271	84	1,441	74

[1] – Aflatoxins contamination level in the fraction ($B_1 + B_2 + G_1 + G_2$); [2] – Relative participation in weight of the fraction; [3] – Relative participation of the fraction in the whole sample contamination; [4] – Estimated contamination.

Table 4. Analysis of the variance.

Variation factors	G.L.	S.Q.	Q.M.	F Value	Prob.>F
Type	1	165.0928534	165.0928534	121.7659	0.00001
Residue	58	78.6376526	1.3558216		
Total	59	243.7305060			

Overall mean value = 3.519069; Variation coefficient = 33.088%.

Table 5. Averages of the factor type.

No. of Treatments	Name	No. of Repetitions	Means	Original means
1	T1	30	1.860290 a	5.925602
2	T2	30	5.177848 b	176.800888

T1 = Sound kernels; T2 = Non-sound kernels.

proportion but much more contaminated, would contribute to reduce the aflatoxin contamination of corn lots bellow the tolerance levels of the Brazilian legislation in 87% of the samples, notwithstanding the Shotwell *et al.* (14,15) findings.

From the results the following conclusion could be drawn: a) The sound kernels fraction has presented the lowest contamination levels in all samples; b) The non-sound kernels fraction, even being small in weight, has contributed with 84% of the estimated contamination of the samples; c) The segregation of the non-sound kernels would favor a reduction in the contamination of the corn lots.

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RESUMO

Distribuição de aflatoxinas em frações de milho segregadas visualmente por defeitos

A distribuição de aflatoxinas em frações de milho obtidas após segregação visual de defeitos em 30 amostras, sabidamente contaminadas, foi estudada. Cada amostra foi passada em peneiras de crivos redondos de 5,0 mm de diâmetro, classificadas por tipo e então separadas em grãos sadios (regulares) e não sadios (ardidos, avariados, brotados, mofados carunchados, chochos, quebrados, descoloridos e avariados por outras causas) definidos pela Classificação Oficial Brasileira para milho. Os grãos não sadios mostraram contaminação maior em todas as amostras. O nível de contaminação dos grãos não sadios (20% do peso total) variou de 23 a 1365 µg/kg de aflatoxinas (B_1, B_2, G_1 e G_2) e foram mais elevados que nos grãos sadios ($p<1\%$), que variaram de não detectada (ND) a 125 µg/kg e, em 87% destas os conteúdos de aflatoxinas foram menores que 20 µg/kg. Foram encontrados índices estatisticamente significativos entre as percentagens de grupos de defeitos, tais como, fermentados, imaturos, mofados, ardidos, brotados, total de grãos avariados, com os níveis estimados de contaminação para as frações sadias e não sadias. Concluiu-se que a fração de grãos não sadios, mesmo apresentando pequeno peso, contribuiu com 84% da contaminação estimada das amostras e que a separação dos grãos não sadios poderá favorecer uma redução na contaminação dos lotes de milho. Os tipos 3 e AP predominaram nas amostras do experimento.

Palavras-chave: aflatoxinas, milho, contaminação, distribuição, separação visual, frações.

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